# EXHIBIT X

# Histology Embedding and Staining Protocol for PROLENE Mesh and Sutures

**First Written : 1/19/2016**
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**PURPOSE:**

The purpose of this document is to describe histology embedding and staining procedures for PROLENE mesh and suture material.

**PROCEDURES:**

A. General

1. Photo documentation
   a. Take photos of each part of this protocol.
   b. Clean, uncontaminated hands/gloves will be used for handling the camera at all times.
2. Reporting and sample management
   a. Cut mesh/suture pieces using a clean razorblade
   b. Enter each sample into Exponent's Evidence Database
   c. Prepare labeled vials for incubating and storing each sample
   d. Record sample names/evidence ID numbers
   e. Record observations throughout the protocol (e.g. sample appearance and changes, solution appearance and changes)
3. Safety
   a. Wear personal eye protection, nitrile gloves, and a lab coat while conducting this experiment

B. Samples to be prepared for histology embedding and staining
   1. Test samples – Segments from each of the following:
      a. 3 PROLENE TVT mesh devices
      b. 1 PROLENE suture
      c. 1 PROLENE hernia mesh

Each sample will have been treated with one of the following conditions:

d. Chemical oxidation (Guelcher protocol)
e. QUV oxidation
f. Serum-coated
g. Control (Pristine)

C. Paraffin-Embedding Protocol[1]
   1. Process and embed samples in an automated tissue processor according to the following schedule:

[1] Paraffin-embedded samples are prepared and stained following the protocol submitted by Dr. Iakovlev.

## Histology Embedding and Staining Protocol for PROLENE Mesh and Sutures



| Processing Step | Incubating Solution | Number of Changes | Duration of Each Incubation Step |
|---|---|---|---|
| 1 | 70% Reagent Alcohol | 2 | 1 hour each |
| 2 | 80% Reagent Alcohol | 1 | 1 hour |
| 3 | 95% Reagent Alcohol | 1 | 1 hour |
| 4 | 100% Reagent Alcohol | 3 | 1.5 hours each |
| 5 | Xylene substitute (ProPar, Manufacturer ) | 3 | 1.5 hours each |
| 6 | Leica EM400 Paraffin wax | 2 | 2 hours each |

2. Embed samples in paraffin blocks using Leica EM400 wax
3. Trim the paraffin blocks as necessary and cut at 4-6 μm-thick sections
4. Briefly float the paraffin sections in a water bath set to 40-45$^{o}$C to remove wrinkles and allow them to flatten
5. Mount the sections onto adhesive-coated glass slides, then air-dry for 30 minutes and bake in a 45-50$^{o}$C oven overnight

D. Staining protocol for paraffin-embedded samples

1. Stain samples using an automated stainer programmed with the following protocol:

| Processing Step | Incubating Solution | Duration of Each Incubation Step |
|---|---|---|
| 1 | 65$^{o}$C | 10 min |
| 2 | Xylene | 3 min |
| 3 | Xylene | 2 min |
| 4 | Xylene | 2 min |
| 5 | 100% Alcohol | 1 min |
| 6 | 100% Alcohol | 1 min |
| 7 | 95% Alcohol | 1 min |
| 8 | Water | 1 min |
| 9 | Harris Hematoxylin | 10 min |
| 10 | Wash station | 1 min |
| 11 | Acid Alcohol | 30 sec |
| 12 | Water | 2 min |

| | | |
|---|---|---|
| 13 | Ammonia Water | 1 min |
| 14 | Water | 1 min |
| 15 | Eosin | 2 min |
| 16 | 100% Alcohol | 1 min |
| 17 | 100% Alcohol | 1 min |
| 18 | 100% Alcohol | 1 min |
| 19 | Xylene | 1 min |
| 20 | Xylene | 1 min |

E. Resin-embedding protocol
1. Process and embed samples in an automated tissue processor according to the following schedule:

| Processing Step | Incubating Solution | Number of Changes | Duration of Each Incubation Step |
|---|---|---|---|
| 1 | 70% Reagent Alcohol | 2 | 1 hour each |
| 2 | 80% Reagent Alcohol | 1 | 1 hour |
| 3 | 95% Reagent Alcohol | 1 | 1 hour |
| 4 | 100% Reagent Alcohol | 3 | 1.5 hours each |
| 5 | Technovit 7200 | 3 | 3 hours each |

2. Polymerize the resin samples using a visible light polymerization unit
3. Trim the blocks as necessary, cut using a diamond saw blade, then ground and polish to approximately 50 µm thickness
4. Stain the resin-embedded samples using an automated stainer programmed with the following protocol:

| Processing Step | Incubating Solution | Duration of Each Incubation Step |
|---|---|---|
| 1 | Water | 1 min |
| 2 | Harris Hematoxylin | 10 min |
| 3 | Water | 1 min |


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| | | |
|---|---|---|
| 4 | Acid Alcohol | 30 sec |
| 5 | Water | 1 min |
| 6 | Ammonia water | 1 min |
| 7 | Water | 1 min |
| 8 | Eosin | 1 min |
| 9 | Water | 30 sec |

Changes to this protocol may be made at the time of the experiment(s) at the judgement of Exponent staff overseeing this experiment and will be documented accordingly.